In the Name of GOD

Ardabil University of Medical Sciences

School of Medicine

A Thesis Submitted to the School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

Title:

Evaluation of the cytotoxic effects and apoptosis induction of Curcumin and it's 2,6-Bis Benzylidine cyclohexanone derivatives on AGS cancer cell line

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Dedication

Dedicated to:

Humanity,

Something that needs to be taken care of more than anything we think.

Acknowledgments

It was such a long journey from the time I started to write my dissertation until the moment I finished defending it. Now in retrospect, I can see what an incredible opportunity I was provided with and how proud I am to be the one who presents this dissertation. Definitely, my efforts and work would not come into a result if there was not the assistance of certain people, so it behooves me to show my deepest appreciation to them.

First, I want to thank my mother for her unconditional love and her caring disposition and the fact that she was always there for me. Needless to say, receiving unwavering supports from my beloved brother and sister was one of the reasons which makes me to continue strongly.

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Still, there is one person that I want to express my eternal gratitude towards him. My father may not be with us today, but I can vividly imagine how proud he could have gotten if he was alive. I hope there was a chance to the expression on his face, though.

Abstract

Background & Objective: Curcumin and its chalcone derivatives inhibit the growth of human cancer cells. It is reported that replacement of two OH groups in curcumin with less polar groups like methoxy increases its anti-proliferative activity. In this study, we explored efficacy of benzylidine cyclohexanone derivatives with non-polar groups, to see if they possess increased anti-cancer activity.

Materials and Methods: Novel 2,6-bis benzylidine cyclohexanone analogues of curcumin were synthesized, and their inhibitory effects on gastric adenocarcinoma (AGS) and esophageal squamous cell carcinoma (KYSE30) cancer cells were studied using an MTT assay. Cell apoptosis was detected by EB/AO staining, and cell cycle was analyzed by flow cytometry. Real-time PCR was performed for gene expression analysis.

Results: All synthesized analogues were cytotoxic toward gastric and esophageal cancer cells and showed lower IC50 values than curcumin. Treatment of 2,6-Bis-(3-methoxy-4-propoxy-benzylidene)-cyclohexanone (BM2) on cells was 17 times more toxic than curcumin after 48 h incubation. All novel compounds were more effective than curcumin in apoptosis induction and cell cycle arrest at G1 phase.

Conclusion: These results suggest that less polar analogues of curcumin have potent cytotoxicity in vitro. However, they need to be investigated further, especially with animal tumor models to confirm their chemotherapeutic activity in vivo.

Keywords: Cytotoxicity, Apoptosis, Cell cycle, Curcumin, Benzylidine cyclohexanone, AGS cells, KYSE30 cells

Figures and Tables

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Abbreviations

Abbreviation	Text
°C	Centigrade
µg	Micro-gram
µl	Micro-liter
AO	Acridine Orange
BAX	bcl-2-like protein 4
Bcl-2	B-cell lymphoma 2
Caspase-3 (CASP3)	**c**ysteine-**asp**artic acid prote**ase** (caspase)
Cm	Centimeter
c-myc	Cellular homolog of the retroviral v-myconcogene
Cyclin D1	Regulator of G1 phase of the cell cycle
DAPI	4',6'-Diamidino-2-Phenylindole
DNA	Deoxyribonucleic Acid
EB	Ethidium Bromide
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
G	Gram

G1	Gap 1 Phase
G2	Gap 2 Phase
GC	Gastric Cancer
GI	Gastrointestinal
H	Hour(s)
IC50	The Half Maximal Inhibitory Concentration
IV	Intravenous
kPa	Kilopascal
Kg	Kilogram
M	Mitosis
Mg	Milligram
Min	Minute
Ml	Milliliter
mol wt	Molecular Weight
MTT	MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide)
NAD(P)H	Nicotinamide Adenine Dinucleotide (Phosphate)
NIH	National Institute of Health
Nm	Nanometer
OD	Optical Density
PBS	Phosphate Buffered Saline

Pg	Pico-gram
Ppm	Parts Per Million
Rcf	Relative Centrifugal Force
Rpm	Round Per Minute
S	Synthesis Phase
SD	Standard Deviation
SEM	Standard Error of the Mean
Survivin	Baculoviral inhibitor of apoptosis repeat-containing 5 or BIRC5
UV	Ultraviolet
VEGFA	Vascular endothelial growth factor A
w/v	Weight/Volume

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